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STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.
1100 NEW YORK AVENUE, N.W.
WASHINGTON, DC 20005

EXAMINER
CHEN, SHIN LIN

ART UNIT	PAPER NUMBER
1632	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/594,188	KOLOSSOV ET AL.	
	Examiner	Art Unit	
	Shin-Lin Chen	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *06 December 2010*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-11,13,17,19-31,45-52,54-67,69 and 70* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-11,13,17,19-31,45-52,54-67,69 and 70* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicant's amendment filed 12-6-10 has been entered. Claims 2, 8, 10, 11, 30, 46 and 66 have been amended. Claims 32 and 68 have been canceled. Claims 1-11, 13, 17, 19-31, 45-52, 54-67, 69 and 70 are pending and under consideration.

Claim Rejections - 35 USC § 112

1. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

2. Claims 1-11, 13, 17, 19-31, 45-52, 54-67, 69 and 70 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for producing embryoid bodies (EBs) from pluripotent embryonic stem (ES) cells, embryonic germ (EG) cells or pluripotent non-embryonic stem cells, does not reasonably provide enablement for a method for producing embyroid bodies (EBs) from multipotent cells, including early primitive ectoderm-like cells, multipotent adult progenitor cells, adult neural stem cells, adult mesenchymal stem cells, ductal stem cells, muscle derived stem cells, hematopoietic stem cells, pancreatic stem cells, follicular stem cells, and any other type of adult stem cells or progenitor cells, and the production of a differentiated cell which is a cardiomyocyte from said EBs. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

While determining whether a specification is enabling, one considered whether the claimed invention provides sufficient guidance to make and use the claimed invention, if not,

whether an artisan would have required undue experimentation to make and use the claimed invention and whether working examples have been provided. When determining whether a specification meets the enablement requirement, some of the factors that need to be analyzed are: the breadth of the claims, the nature of the invention, the state of the prior art, the level of one of ordinary skill, the level of predictability in the art, the amount of direction provided by the inventor, the existence of working examples, and whether the quantity of any necessary experimentation to make or use the invention based on the content of the disclosure is "undue" (In re Wands, 858 F.2d at 737, 8 USPQ2d 1400, 1404 (Fed. Cir.1988)).

Furthermore, the USPTO does not have laboratory facilities to test if an invention with function as claimed when working examples are not disclosed in the specification, therefore, enablement issues are raises and discussed based on the state of knowledge pertinent to an art at the time of the invention, therefore skepticism raised in the enablement rejections are those raised in the art by artisans of expertise.

Claims 1-11, 13, 17, 19-31, 45-52, 54-67, 69 and 70 are directed to a method for producing embryoid bodies (EBs) from pluripotent cells, such as murine embryonic stem cells, comprising obtaining a liquid single cell suspension culture of pluripotent cells, collecting and suspending the cells in a container to a density of about $0.5x10^6$ to $5x10^6$ cells/ml or $0.1x10^6$ to $1x10^6$ cells/ml, rocking the container containing the liquid single cell suspension culture of pluripotent cells until generation of cell aggregates, rocking the container until formation of EBs, wherein the final concentration of EBs is about 500 EBs/ml, or diluting the resulting EBs to a concentration of about 100-2000 EBs/10 ml. Claims 5 and 49 specify the culture medium is IMDM with 20% FCS and 5% CO2. Claims 6 and 50 specify the culture condition comprises

37oC and 95% humidity. Claims 7-9 specify the culture of pluripotent cells has a concentration of $1x10^6$ to $5x10^6$ cells/ml and the suspension is cultured for about 6 and 16-20 hours, respectively. Claim 10 specifies the suspension is cultured in T25 flasks. Claim 11 specifies the dilution in step (iv) is 1:10. Claims 51 and 52 specify the culture of pluripotent cells has a concentration of $0.1x10^6$ to $0.5x10^6$ cells/ml and the suspension is cultured for about 48 hours, respectively. Claims 17 and 54 further comprise culturing EBs under conditions allowing differentiation of the EBs into cardipmyocytes. Claims 19 and 55 further comprise selection of cardiomyocytes by use of one or more selectable markers or agents or both. Claims 20-28 and 56-64 specify the cells are genetically engineered using a selectable marker, such as puromycin resistant gene, and/or a reporter gene, such as EGFP, under the control of a cell type-specific regulatory sequence. Claims 29, 30, 65, 66, 69 and 70 specifies the cell type-specific regulatory sequence is atrial- and/or ventricular-specific and is selected from promoters of alphaMHC or MLC2v. Claims 31 and 67 read on embryoid body obtained from the claimed method.

The specification discloses transfection of mouse embryonic stem cells with palphaMHC-GFP vector comprising GFP gene under the control of the cardiac alpha-myosin heavy chain (alpha-MHC) promoter. The ES cells were cultured on 10 cm Petri dishes in the presence of 15% FCS and $1x10^3$ U/ml LIF on a layer of feeder cells (inactivated mouse embryonic fibroblasts). The ES cells were trypsinized, centrifuged, and resuspended in IMDM medium with 20% FBS, and the ES cells were cultured in suspension at a density of $2x10^6$ cells/ml in a 6 cm Petri dish in IMDM with 20% FCS on a rocking table with or without agitation. EBs in suspension was transferred to a COPAS select particle sorter and the EBs were cultured in

IMDM and 20% FCS, and fluorescent areas representing cardiac cells were detected by fluorescent microscopy (e.g. Examples 1 and 2).

The specification fails to specifically define the phrase "pluripotent cells". The phrase "pluripotent cells" can encompass embryonic stem cells, embryonic germ cells, early primitive ectoderm-like cells, multipotent adult progenitor cells, adult neural stem cells, adult mesenchymal stem cells, ductal stem cells, muscle derived stem cells, hematopoietic stem cells, pancreatic stem cells, follicular stem cells, and any other type of adult stem cells or progenitor cells. The specification fails to provide adequate guidance and evidence for how to produce EBs from numerous different pluripotent cells with the final concentrations of 500 EBs/ml or 100-2000 EBs/10ml.

A search of the state of the art of generating EBs, it is apparent that only pluripotent embryonic stem cells or embryonic germ cells can produce EBs under a certain culturing condition. Kurosawa, H., 2007 (Journal of Bioscience and Bioengineering, Vol. 5, p. 389-398) reports that embryonic stem (ES) cells cultured in suspension in the absence of LIF or MEF feeder layers would spontaneously differentiate and form three-dimensional multicellular aggregates called embryoid bodies (EBs). "EBs recapitulate many aspects of cell differentiation during early embryogenesis, and play an important role in the differentiation of ES cells into a variety of cell types in vitro" (e.g. abstract, p. 389, left column). "EB formation has been utilized widely as a trigger of in vitro differentiation of both mouse and human ES cells" (e.g. p. 389, right column). No literature has reported formation of EBs from various pluripotent cells, such as early primitive ectoderm-like cells, multipotent adult progenitor cells, adult neural stem cells, adult mesenchymal stem cells, ductal stem cells, muscle derived stem cells, hematopoietic stem

cells, pancreatic stem cells, follicular stem cells, or any other type of adult stem cells or progenitor cells. There is no evidence of record that demonstrate the formation of EBs from various pluripotent cells in vitro or in vivo. Absent specific guidance, one skilled in the art at the time of the invention would not know how to form EBs from numerous different pluripotent cells in vitro or in vivo.

Applicant cites WO03/018780, Abuljadayel et al., and WO03/052080 in the amendment filed 3-11-09 and points out that non-embryonic stem cells also can form EBs. It is noted that those references show formation of EBs from de-differentiated pluripotent stem cells derived from somatic cells, undifferentiated stem cells, and pluripotent non-embryonic stem cells, respectively. None of the reference shows formation of EBs from early primitive ectoderm-like cells, multipotent adult progenitor cells, adult neural stem cells, adult mesenchymal stem cells, ductal stem cells, muscle derived stem cells, hematopoietic stem cells, pancreatic stem cells, follicular stem cells, and any other type of adult stem cells or progenitor cells. It was unpredictable at the time of the invention whether various "pluripotent cells" could form EBs in the process of differentiating into corresponding differentiated cell linage.

For the reasons set forth above, one skilled in the art at the time of the invention would have to engage in undue experimentation to practice over the full scope of the invention claimed. This is particularly true based upon the nature of the claimed invention, the state of the art, the unpredictability found in the art, the teaching and working examples provided, the level of skill which is high, the amount of experimentation required, and the breadth of the claims.

Claim Rejections - 35 USC § 112

3. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

4. Claims 9 and 10 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The phrase "wherein the suspension is cultured for about 16 to 20 hours" in claim 9 is vague and renders the claim indefinite. Claim 9 depends from claim 8, which limits the suspension is cultured for about 6 hours. Claim 9, the dependent claim, has a broader limitation than the claim it depends from. It is unclear how the suspension is cultured for about 16 to 20 hours when the suspension is cultured for about 6 hours. Claim 10 depends from claim 9.

Claim Rejections - 35 USC § 102

5. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

6. Claims 31 and 67 are rejected under 35 U.S.C. 102(e) as being anticipated by Dang et al., June 26, 2003 (US 2003/0119107 A1, IDS).

Claims 31 and 67 are directed to an embryoid body obtained by the claimed method of claim 1 or claim 45.

Dang teaches a novel bioprocess where aggregation of ES cells and EBs are controlled, and the EBs can be generated with high efficiency and cultured in high cell density and well-mixed system (e.g. abstract). Some ES cell lines require aggregation of multiple ES cells to enable EB formation and the EB can be further differentiated into differentiated embryonic stem cells and/or tissue, such as cardiomyocytes or cardiac tissue (e.g. [0020]). The invention is not limited to ES cell but also include any spheroid forming cell type, such as adult pluripotent cells, embryonic germ cells, early primitive ectoderm-like cells and neuronal stem cells (e.g. [0016]). The ES cells are encapsulated to control cell aggregation such that each capsule gives rise to one embryoid body and each capsule contains a predetermined number of ES cells that are permitted to aggregate to from a single EB (e.g. [0019]). Dang teaches single cell liquid suspension cultures (scLSC) of ES cells (e.g. [0025], [0028]). Dang teaches a method for efficient formation of EBs and the culture of EBs in suspension at higher cell densities by controlling cell aggregation (e.g. [0051]). A method of culturing spheroid-forming cells, such as pluripotent cells in a bioreactor system where the culture conditions can be measured and controlled (e.g. [0052]). The bioreactor or culture system keeps the cells and/or spheroids in liquid suspension by stirring, but other methods or means, such as agitation of the system, can be used (e.g. [0053]). Dang shows EB efficiency in liquid culture (LSC) is 42+_9% and 8.5+_2.7% with starting ES cells at 10^3 and 10^4 cells/ml, respectively (e.g. Table 4). The EB derived from murine ES cells generated by Dang would be the same as the EB derived from murine ES cells generated by the claimed method of the instant invention. The claims are product claims.

Although the method of obtaining the product is different, however, the product would be the same because they both derived from the same murine ES cells. Thus, the claims are anticipated by Dang.

Claim Rejections - 35 USC § 103

7. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

8. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

9. Claims 45-52, 54-66 and 70 are rejected under 35 U.S.C. 103(a) as being unpatentable over Dang et al., June 26, 2003 (US 20030119107 A1, IDS) in view of Yan et al., February 2003 (US 20030027331 A1) and Kehat et al., 2001 (The Journal of Clinical Investigation, Vol. 108, No. 3, p. 407-414, IDS).

Claims 45-52, 54-66 and 70 are directed to a method for producing embryoid bodies (EBs) from pluripotent cells, such as embryonic stem cells, comprising obtaining a liquid single

cell suspension culture of pluripotent cells, collecting and suspending the cells in a container to a density of about $0.1x10^6$ to $1x10^6$ cells/ml, rocking the container containing the liquid single cell suspension culture of pluripotent cells until generation of cell aggregates, rocking the container until formation of EBs, and diluting the resulting EBs to a concentration of about 100-2000 EBs/10 ml. Claim 49 specifies the culture medium is IMDM with 20% FCS and 5% CO2. Claim 50 specifies the culture condition comprises 37oC and 95% humidity. Claims 51 and 52 specify the culture of pluripotent cells has a concentration of $0.1x10^6$ to $0.5x10^6$ cells/ml and the suspension is cultured for about 48 hours, respectively. Claim 54 further comprises culturing EBs under conditions allowing differentiation of the EBs into cardipmyocytes. Claim 55 further comprises selection of cardiomyocytes by use of one or more selectable markers or agents or both. Claims 56-64 specify the cells are genetically engineered using a selectable marker, such as puromycin resistant gene, and/or a reporter gene, such as EGFP, under the control of a cell type-specific regulatory sequence. Claims 65, 66 and 70 specifies the cell type-specific regulatory sequence is atrial- and/or ventricular-specific and is selected from promoters of alphaMHC or MLC2v.

Dang teaches a novel bioprocess where aggregation of ES cells and EBs are controlled, and the EBs can be generated with high efficiency and cultured in high cell density and well-mixed system (e.g. abstract). Some ES cell lines require aggregation of multiple ES cells to enable EB formation and the EB can be further differentiated into differentiated embryonic stem cells and/or tissue, such as cardiomyocytes or cardiac tissue (e.g. [0020]). The invention is not limited to ES cell but also include any spheroid forming cell type, such as adult pluripotent cells, embryonic germ cells, early primitive ectoderm-like cells and neuronal stem cells (e.g. [0016]).

The ES cells are encapsulated to control cell aggregation such that each capsule gives rise to one embryoid body and each capsule contains a predetermined number of ES cells that are permitted to aggregate to from a single EB (e.g. [0019]). Dang teaches single cell liquid suspension cultures (scLSC) of ES cells (e.g. [0025], [0028]). Dang teaches a method for efficient formation of EBs and the culture of EBs in suspension at higher cell densities by controlling cell aggregation (e.g. [0051]). A method of culturing spheroid-forming cells, such as pluripotent cells in a bioreactor system where the culture conditions can be measured and controlled (e.g. [0052]). The bioreactor or culture system keeps the cells and/or spheroids in liquid suspension by stirring, but other methods or means, such as agitation of the system, can be used (e.g. [0053]). Dang also teaches individual R1 ES cells expressing GFP protein under the control of a constitutively active promoter was encapsulated with a cyan labeled ES cell, and the two cell types survived and proliferated to form spheroid containing two sources of cells (chimeric spheroids). The technology of forming chimeric spheroids can be used to manipulate the differentiation of the pluripotent cells into specific types of tissue using cell specific signals (e.g. [0185]). Dang teaches introduction of a specific construct expressing a marker gene under the control of a cell or tissue specific promoter into ES cells such that the transfected ES cells can be expanded, differentiated and selected to generate the desired cell type of interest (e.g. [0076], [0089]). The cell lineage-specific promoter for cardiomyocytes is alpha-cardiac myosin heavy chain promoter and MLC2v (e.g. Table 9). The reporter gene could be Hygromycin resistance gene, the puromycin resistance gene or G418 resistance gene (e.g. [0094]). Dang further teaches culturing CCE murine embryonic stem cell in IMDM medium supplemented with 15% FBS at 37oC in humidified air with 5% CO2 (e.g. [0110]). Dang shows EB efficiency in liquid culture

(LSC) is 42+_9% and 8.5+_2.7% with starting ES cells at 10^3 and 10^4 cells/ml, respectively (e.g. Table 4). The EB efficiency of 8.5% of 10^4 cells/ml accounts for about 850 EBs/ml. Even if there is only 1% EB efficiency when the starting ES cells is 10^5 cells/ml in liquid cell culture, the resulting EB would be about 100 EBs/ml, which is well within the range of claim 45.

Dang does not specifically teach rocking single cell suspension of the pluripotent cells or the cell concentration of about $0.1x10^6$ to $1x10^6$ cells/ml, 1:10 dilution, and culturing the suspension culture for about 6hr or 16-20hr, 20% FCS and 95% humidity. Dang also does not specifically teach the marker gene and reporter gene are contained on the same recombinant nucleic acid molecule or on the same cistron.

Yan discloses that homozygous stem (HS) cells are pluripotent cells (e.g. [0016]) and teaches trypsinizing the HS cells grow the HS cells in single cell suspension culture in 2 ml ES-LIF medium and culturing the cells as suspension cells in suspension culture at a density of $1-3x10^6$ cells to allow stem cells to form rounded spherical clusters known as embryoid bodies (EBs) for 4-6 days (e.g. [0293]). The isolated HS cells can be induced to differentiated into cardiomyocytes using technique known in the art such as Kehat (2001) (e.g. [0220]).

Kehat teaches culturing human ES cells in suspension and plated to form EBs, and spontaneously contracting areas appeared in 8.1% of EBs. Cells from said contracting area within EBs were stained positively with anti-cardiac myosin heavy chain, anti-alpha-actinin, anti-desmin, anti-cardiac troponin 1 and anti-ANP antibodies. The human ES cell-derived cardiomyocytes displayed structural and functional properties of early stage cardiomyocytes (e.g. abstract). ES cell clumps were grown in plastic petri dishes at a cell density of about $5x10^6$ cells in a 58 mm dish (e.g. p. 408, bridging left and right column).

It would have been prima facie obvious for one of ordinary skill in the art at the time of the invention to rock pluripotent cells in single cell suspension because Dang teaches single cell liquid suspension cultures (scLSC) of ES cells and a bioreactor or culture system that keeps the cells and/or spheroids in liquid suspension by stirring or other methods or means, such as agitation of the system. Rocking is a type of agitation (see specification [0043], "Agitating the suspension for about six hours on a rocking table", or the amendment filed 9-28-09, page 14, 2nd full paragraph, "the agitation (e.g. rocking)". Therefore, it would be obvious to one of ordinary skill in the art to rock the single cell suspension of the ES cells taught by Dang. It also would have been prima facie obvious for one of ordinary skill in the art at the time of the invention to culture the ES cells at a concentration of $0.5x10^6$ to $5x10^6$ cells/ml or about $0.1x10^6$ to $1x10^6$ cells/ml, 1:10 dilution, with a certain final concentration of EBs in the suspension culture, and culturing the suspension culture for about 6hr or 16-20hr because Yan teaches growing pluripotent cells at a concentration of $1\text{-}3x10^6$ cells/2ml and Kehat teaches culturing ES cells at about $5x10^6$ cells in a 58 mm dish. The concentration of $1\text{-}3x10^6$ cells/2ml is within the range of $0.5x10^6$ to $5x10^6$ cells/ml or about $0.1x10^6$ to $1x10^6$ cells/ml. Determining effective dose or conditions for culturing ES cells is routine optimization of a result-effective variable and is obvious to one of ordinary skill. Dang teaches a method of culturing spheroid-forming cells, such as pluripotent cells in a bioreactor system where the culture conditions can be measured and controlled. Culturing ES cells at these conditions or with certain final concentration of EBs would be obvious to one of ordinary skill in order to optimize the culture condition for the ES cells. Having the marker gene and reporter gene contained on the same recombinant nucleic acid molecule or on the same cistron would be obvious to one of ordinary skill because determining

effective orientation of the marker gene and reporter gene is routine optimization of a result-effective variable and is obvious to one of ordinary skill. One of ordinary skill would orient the marker gene and reporter gene in a vector in order to optimize the expression of the marker gene and reporter gene at target cells.

It would have been prima facie obvious for one of ordinary skill in the art to culture ES cells in medium having 20% FCS and 95% humidity because Dang teach culturing ES cells in 15% FBS and humidified condition. FBS and FCS are the same and growing ES cells in 95% humidity would be obvious to one of ordinary skill in order to optimize the culture condition for the ES cells. Determining effective dose or conditions for culturing ES cells is routine optimization of a result-effective variable and is obvious to one of ordinary skill.

The final concentration of EBs/ml is further discussed as followed. Claim 45 reads on diluting the resultant EBs to a concentration of about 100-2000 EBs/10 ml, which is about 10-200 EBs/ml final concentration. As discussed above, Dang shows EB efficiency in liquid culture (LSC, static culture) is 42+_9% and 8.5+_2.7% with starting ES cells at 10^3 and 10^4 cells/ml, respectively (e.g. Table 4). The EB efficiency of 42% of 10^3 ES cells/ml accounts for about 420 EBs/ml. The EB efficiency of 8.5% of 10^4 cells/ml accounts for about 850 EBs/ml. Although in LSC method the EB efficiency decreases when the starting ES cell number increases, however, the resulting EBs/ml still increases from 420 EBs/ml to 850 EBs/ml. Even if there is only 1% EB efficiency when the starting ES cells is 10^5 cells/ml in liquid cell culture, the resulting EB would be about 100 EBs/ml, which is well within the range of claim 45. Although rocking the cell culture (instant invention) is different from LSC (static) method, rocking the cell culture might provide more shear press and increase collision between the forming EBs, however, it is

conceivable that rocking the ES cell culture at starting concentration of $0.1x10^6$ to $1x10^6$ cells/ml could result in final concentration of EBs at more than 10 EBs/ml. It is noted that in the Declaration filed 9-28-09, page 3, applicant points out that $0.2x10^6$ ES cells/ml in 250 spinner flask using the spinner method as taught by Hescheler results in about half the amount of EBs/ml generated by the instant claimed method, which would be about 250-500 EBs/ml. In the amendment filed 2-23-10, page 7, 2nd paragraph, applicant also points out that in previous methods for the production of embryoid bodies the yield of embryoid bodies was in the range of 50/ml. Since the conventional method of forming EBs can produce EBs in the range of 50/ml and rocking the ES cell culture appears to be more advantageous than the conventional methods, it would be prima facie obvious for one of ordinary skill in the art at the time of the invention to produce EBs in the range of 10-200 EBs/ml by using the instant claimed method.

One having ordinary skill in the art at the time the invention was made would have been motivated to do so in order to form EBs from pluripotent cells and to manipulate the differentiation of the pluripotent cells into specific types of cell or tissue using cell specific promoter as taught by Dang, to form EBs from HS cells as taught by Yan or to form EBs from human ES cells as taught by Kehat with reasonable expectation of success.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Shin-Lin Chen whose telephone number is (571) 272-0726. The examiner can normally be reached on Monday to Friday from 9:30 am to 6 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Peter Paras can be reached on (571) 272-4517. The fax phone number for this group is (571) 273-8300.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

Patent applicants with problems or questions regarding electronic images that can be viewed in the Patent Application Information Retrieval system (PAIR) can now contact the USPTO's Patent Electronic Business Center (Patent EBC) for assistance. Representatives are available to answer your questions daily from 6 am to midnight (EST). The toll free number is (866) 217-9197. When calling please have your application serial or patent number, the type of document you are having an image problem with, the number of pages and the specific nature of the problem. The Patent Electronic Business Center will notify applicants of the resolution of the problem within 5-7 business days. Applicants can also check PAIR to confirm that the problem has been corrected. The USPTO's Patent Electronic Business Center is a complete service center supporting all patent business on the Internet. The USPTO's PAIR system provides Internet-based access to patent application status and history information. It also enables applicants to view the scanned images of their own application file folder(s) as well as general patent information available to the public.

For all other customer support, please call the USPTO Call Center (UCC) at 800-786-9199.

Shin-Lin Chen
/Shin-Lin Chen/
Primary Examiner
Art Unit 1632